

CLAIMS

1. An anti-proBNP(1-108) antibody, characterized in that, firstly, it specifically recognizes the sequence RAPR₇₆S₇₇P (SEQ ID No. 5) of proBNP(1-108) and does not substantially recognize the peptides BNP(1-76) or BNP(77-108) and, secondly, it has the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples.
2. The anti-proBNP(1-108) antibody as claimed in claim 1, which specifically recognizes the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅ (SEQ ID No. 4) of proBNP(1-108).
3. The anti-proBNP(1-108) antibody as claimed in claim 1, which specifically recognizes the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108) of proBNP(1-108).
4. A method for obtaining an anti-proBNP(1-108) antibody as defined in one of claims 1, 2 and 3, in which an animal is immunized with the whole proBNP(1-108) molecule, and then the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.
5. A method for obtaining an anti-proBNP(1-108) antibody as defined in one of claims 1, 2 and 3, in which an animal is immunized with a peptide chosen from - a peptide of formula
- $$a_1-X_1-RAPRSP-X_2-a_2 \quad (I)$$
- where
- a₁ may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,
- X₁ represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X₂ represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

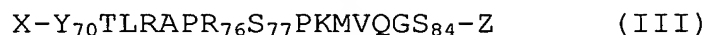
a₂ may represent an OH function, an NH₂ function or an alkoxy group;

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the sequence

X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅-Z (II) or from the sequence

X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄-Z (III) by substitution of one or more among the amino acids Y₇₀, T₇₁, L₇₂, K₇₉, M₈₀, V₈₁, Q₈₂, G₈₃, S₈₄ and G₈₅, with it being possible for X to be or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);

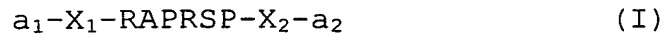
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 109);

and, optionally, the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

6. A method for obtaining a hybridoma that secretes an anti-proBNP(1-108) antibody as defined in one of

claims 1, 2 and 3, in which an animal is immunized with a peptide chosen from

- a peptide of formula



5 where

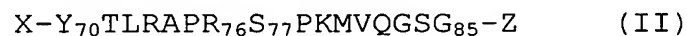
a_1 may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

10 X_1 represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X_2 represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural
15 sequence of proBNP(1-108),

a_2 may represent an OH function, an NH_2 function or an alkoxyl group;

- a peptide of formula



20 where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

25 - a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an
30 OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the sequence

$X-Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}-Z$ (II) or from the sequence

35 $X-Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}-Z$ (III) by substitution of one or more among the amino acids Y_{70} , T_{71} , L_{72} , K_{79} , M_{80} , V_{81} , Q_{82} , G_{83} , S_{84} and G_{85} , with it being possible for X to be or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108),

and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);
5 - the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 109);
immunoglobulin-secreting lymphocytes are taken from this animal,
and the lymphocytes are fused with myeloma cells so as
10 to obtain at least one immunoglobulin-secreting hybridoma.

7. The method as claimed in either of claims 5 and 6, in which the peptide of formula (II) has the sequence
15 Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅ (SEQ ID No. 4).

8. The method as claimed in either of claims 5 and 6, in which the peptide of formula (III) has the sequence
20 Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108).

9. A hybridoma which can be produced by the method as claimed in one of claims 6, 7 and 8.

10. An anti-proBNP(1-108) monoclonal antibody secreted
25 by a hybridoma as claimed in claim 9.

11. A method of *in vitro* diagnosis of heart failure in a human, comprising bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined
30 in one of claims 1, 2, 3 and 10, and detecting the proBNP(1-108) in the sample.

12. A method of *in vitro* diagnosis of heart failure in a human, comprising:
35 a) bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined in one of claims 1, 2, 3 and 10,
b) incubating the mixture under conditions that allow the formation of antigen-antibody complexes, and

c) revealing the antigen-antibody complexes formed, optionally using a labeled detection antibody capable of binding specifically to the proBNP(1-108) present in the primary complex, or using a labeled detection antigen capable of binding to the antibody directed against said proBNP(1-108) present in the primary complex.

13. The method of diagnosis as claimed in claim 12, which also comprises a step d) for correlating the amount of antigen-antibody complexes revealed with the clinical condition of the individual.

14. A kit for detecting proBNP(1-108) in a biological sample, containing at least one antibody as defined in one of claims 1, 2, 3 and 10.

15. The kit for detecting proBNP(1-108) in a biological sample, as claimed in claim 14, containing:

(i) in a container, at least one antibody as defined in any one of claims 1, 2, 3 and 10;
(ii) in another container, at least one peptide chosen from

- a peptide of formula
25 $a_1-X_1-RAPRSP-X_2-a_2$ (I)
where

a_1 may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

30 X_1 represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X_2 represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

35 a_2 may represent an OH function, an NH_2 function or an alkoxyl group;

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an
5 OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula



where X may be H or may represent either an acetyl
10 group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the
15 sequence

X-Y₇₀TLRAPR₇₆S₇₇PKMVQSG₈₅-Z (II) or from the sequence
X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄-Z (III) by substitution of one
or more among the amino acids Y₇₀, T₇₁, L₇₂, K₇₉, M₈₀, V₈₁,
Q₈₂, G₈₃, S₈₄ and G₈₅, with it being possible for X to be
20 or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

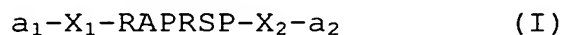
- the peptide having the sequence
25 Y₇₀TLRAPR₇₆S₇₇PKMVQSG₈₅ (SEQ ID No. 4);

- the peptide having the sequence
Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 109);

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);

30 - the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 108).

16. A peptide of formula:



35 where

a₁ may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group or an acetyl group,

X₁ represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

5 X₂ represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a₂ may represent an OH function, an NH₂ function, or an alkoxy group.

10 17. A peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an
15 OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

18. The peptide as claimed in claim 17, having the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQSGS₈₅ (SEQ ID No. 4).

20

19. A peptide of formula:



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the
25 sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

20. The peptide as claimed in claim 19, having the
30 sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108).

21. A peptide comprising a sequence derived from the sequence X-Y₇₀TLRAPR₇₆S₇₇PKMVQSGS₈₅-Z (II) or from the sequence X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄-Z (III) by substitution
35 of one or more among the amino acids Y₇₀, T₇₁, L₇₂, K₇₉, M₈₀, V₈₁, Q₈₂, G₈₃, S₈₄ and G₈₅, with it being possible for X to be or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1

to 3 amino acids not belonging to the sequence of proBNP(1-108).

22. The peptide as claimed in claim 16, having a
5 sequence chosen from the group consisting of the following sequences

SEQ ID No. 16: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G
(peptide C13P30)

SEQ ID No. 109: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S
10 (peptide CN32)

SEQ ID No. 6: C-G-R-A-P-R-S-P

SEQ ID No. 7: Acetyl-C-G-R-A-P-R-S-P

SEQ ID No. 8: C-G-R-A-P-R-S-P-K

SEQ ID No. 9: Acetyl-C-G-R-A-P-R-S-P-K

15 SEQ ID No. 10: C-G-R-A-P-R-S-P-K-M-V

SEQ ID No. 11: C-G-R-A-P-R-S-P-K-M-V-Q-G-S-G

SEQ ID No. 12: R-A-P-R-S-P-G-C

SEQ ID No. 13: Acetyl-R-A-P-R-S-P-G-C

SEQ ID No. 110: C-Y-T-L-R-A-P-R-S-P-K

20 SEQ ID No. 111: C-Y-T-L-R-A-P-R-S-P-K-M-V

SEQ ID No. 112: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q

SEQ ID No. 113: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G

SEQ ID No. 19: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-βA

SEQ ID No. 20: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-A-T-βA

25 SEQ ID No. 114: Acetyl-C-T-L-R-A-P-R-S-P-K-M-V-Q

SEQ ID No. 115: C-T-L-R-A-P-R-S-P-K-M-V-Q-G

SEQ ID No. 116: C-T-L-R-A-P-R-S-P-K-M-V-Q-G-S

SEQ ID No. 117: C-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G

SEQ ID No. 118: C-L-R-A-P-R-S-P-K-M-V

30 SEQ ID No. 119: C-L-R-A-P-R-S-P-K-M-V-Q

SEQ ID No. 120: L-R-A-P-R-S-P-K-M-V-Q-C

SEQ ID No. 121: C-L-R-A-P-R-S-P-K-M-V-Q-G-S

SEQ ID No. 122: C-L-R-A-P-R-S-P-K-M-V-Q-G-S-G

35 23. A method for obtaining anti-proBNP(1-108) antibodies that specifically recognize the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQSG₈₅, the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ and/or the sequence RAPR₇₆S₇₇P of proBNP(1-108) with the substantial exclusion of the

BNP(1-76) and BNP(77-108) peptides, and that have the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples, in which method an animal is immunized with a peptide as defined in one of claims 16 to 22,

and, optionally, the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

24. A method for obtaining a hybridoma that secretes an anti-proBNP(1-108) antibody that specifically recognizes the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}$, the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}$ and/or the sequence $RAPR_{76}S_{77}P$ of proBNP(1-108) with the substantial exclusion of the BNP(1-76) and BNP(77-108) peptides, and that have the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples, in which method an animal is immunized with a peptide as defined in one of claims 16 to 22, immunoglobulin-secreting lymphocytes are removed from this animal, and the lymphocytes are fused with myeloma cells so as to obtain at least one immunoglobulin-secreting hybridoma.

25. An anti-proBNP(1-108) antibody, characterized in that it is obtained by a method as claimed in claim 23.

26. The anti-proBNP(1-108) antibody as claimed in claim 25, which specifically recognizes the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}$ or the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}$ of proBNP(1-108).

27. A hybridoma which can be produced by the method as claimed in claim 24.

28. An anti-proBNP(1-108) monoclonal antibody secreted by a hybridoma as claimed in claim 27.

29. The anti-proBNP(1-108) monoclonal antibody as claimed in claim 28, secreted by the hybridoma 3D4 deposited with the CNCM under the No. CNCM I-3073.

5 30. A method of *in vitro* diagnosis of heart failure in a human, comprising bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined in any one of claims 25, 26, 28 and 29, and detecting the proBNP(1-108) in the sample.

10

31. A method of *in vitro* diagnosis of heart failure in a human, comprising:

a) bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined in one of claims
15 25, 26, 28 and 29,

b) incubating the mixture under conditions that allow the formation of antigen-antibody complexes, and

c) revealing the antigen-antibody complexes formed, optionally using a labeled detection antibody capable
20 of binding specifically to the proBNP(1-108) present in the primary complex, or using a labeled detection antigen capable of binding to the antibody directed against said proBNP(1-108) present in the primary complex.

25

32. The method of diagnosis as claimed in claim 31, which also comprises a step d) for correlating the amount of antigen-antibody complexes revealed with the clinical condition of the individual.

30

33. A kit for detecting proBNP(1-108) in a biological sample, containing at least one antibody as defined in one of claims 25, 26, 28 and 29.

35 34. A kit for detecting proBNP(1-108) in a biological sample, containing, as standard and/or control, at least one peptide as defined in one of claims 16 to 22.

35. A kit for detecting proBNP(1-108) in a biological sample, containing:

- in a container, at least one antibody as defined in one of claims 25, 26, 28 and 29;
- 5 - in another container, at least one peptide as defined in one of claims 16 to 22.